December, 1916 BOTANICAL SERIES. VOL. VIII, No. 6.

MEMOIRS OF THE DEPARTMENT OF AGRICULTURE IN INDIA

PHYTOPHTHORA ON *VINCA ROSEA*

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PRINTED AND PUBLISHED FOR

THE IMPERIAL DEPARTMENT OF AGRICULTURE IN INDIA

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[Received for publication on the 19th July, 1916.]

At Pusa (India) the latter part of May 1913 was wet, so also most of the month of June. During this period the weather remained cloudy, and the atmosphere was charged with a great deal of moisture. These were ideal conditions for parasitic fungi to flourish to the detriment of their host plants. One of the garden plants that suffered during this period, was *Vinca rosea* belonging to the *N. O. Apocynaceæ*.

Leaves, growing points, tender stems just below growing points, flowers and fruits were observed to damp off, to turn brown and then black. Microscopic examination showed the presence of a parasite of the genus *Phytophthora*. The disease was prevalent for about a fortnight only. It disappeared soon after the weather cleared up and bright sunshine once again flooded the gardens. Even when the weather was wet, if diseased plants were removed to a dry place, the disease made no headway and the plant dropped its diseased parts and brought forth new healthy shoots. The causal parasite has never been found to be of a virulent character. Healthy plants inoculated with pure cultures in the laboratory invariably gave negative results, except when the surrounding atmosphere was kept saturated with moisture, which condition was secured by covering the inoculated plants grown in pots with bell jars with an inner lining of blotting paper and keeping the pots in a basin of water. Under these conditions the effects of the inoculations were visible in about a couple of days by the inoculated parts, flowers, leaves, growing points, stem just underneath the growing point, and fruits, turning brown and then black as in nature. Mature stems could not be successfully inoculated even in such an atmosphere. Follicles, sterilized by washing in corrosive sublimate (1 in 1,000) for five minutes, and unsterilized follicles were placed in six sterilized tubes containing moist cotton plugs. Bits of agar medium containing living mycelium from a healthy culture were used in inoculating these follicles.

Similarly sterilized follicles were placed in water in watch glasses, and were kept in moist chambers under aseptic conditions. These follicles were inoculated by zoospores from the same culture. Those in the tubes did not take the inoculation, even though in many cases aerial mycelium was found growing from the bits of agar medium used for inoculating the follicles; while those left in water showed signs of successful inoculations in twenty-four hours by the rotting that had set in; an aerial growth of mycelium soon followed. These inoculation experiments sufficiently show the weak parasitism of the fungus on *Vinca rosea*. Perhaps this is the only strain as yet isolated from any species of *Phytophthora* having such a weak parasitism.

On diseased leaves, sporangia were formed on both the surfaces, borne on short or long sporangiophores (from 3·4 to over 375μ in length) and emerging as a rule singly through stomata; in rare cases, sporangiophores have been found to be branched; two or three emerging together from a single stoma have also been observed. The sporangiophore, in a few cases, has been found to make its way out by rupturing a guard cell. Sporangia are sometimes borne on such short stalks that they almost seem to be sessile. From petioles and stems sporangiophores emerge singly or in clusters through any part of the epidermis as in *Ph. parasitica* Dast.[1] Sporangia are generally pear-shaped, but often irregular ones have been found. The mycelium within the tissues is both inter and intra-cellular. Haustoria have not been observed.

This *Phytophthora* was easily taken in pure culture. Diseased leaves and follicles, washed with corrosive sublimate (1 in 1,000) for five minutes and then with sterilized water, were incubated and gave, after a few days, a woolly growth. From this pure cultures were obtained by inoculating tubes of Quaker-Oat agar; this medium was prepared as recommended by Pethybridge and Murphy.[2] Also seeds removed aseptically from diseased follicles similarly sterilized on the surface, were introduced in tubes containing nutrient agar and subsequently gave pure cultures of the fungus.

The growth of this fungus on artificial media is very similar to that of *Ph. parasitica*[1] on castor, and also the vegetative, asexual and sexual reproductive organs; therefore a detailed description is not needed.

[1] Dastur, J. F., on *Phytophthora parasitica* nov. spec. *Mem. Dept. Agric. India, Bot. Ser.*, V, No. 4, 1913.

[2] Pethybridge, G. H. and Murphy, P. A., On Pure Cultures of *Ph. infestans* de Bary, and the Development of Oospores. *Sc. Proc. Roy. Dub. Soc.*, XIII (N.S.), No. 36, 1913.

The thickening, commonly found on septa of *Ph. Colocasiæ* Rac.[1] and *Ph. parasitica* Dast. is usually wanting. Very often branches have been found arising through septa, growing for some distance within the empty cells outside the septa and then coming out by perforating the wall as in *Ph. parasitica* and *Fusarium tuberivorum* Wilcox and Link[2] (Fig. 2). Such branches have been observed to form sporangia outside the parent hypha.

Sporangia and zoospores agree in measurements with those of the castor fungus. The formation of secondary sporangia in the *Vinca* fungus is common. They are either stalked or sessile. Sessile secondary sporangia have always been found to arise from the papilla, but stalked ones arise not necessarily from this place.

Zoospores sown in water, after coming to rest, germinate in less than half an hour by giving out one or as many as four germ-tubes. The fungus upon castor has not hitherto been observed to give more than two germ-tubes. The germ-tube either grows into a thin hypha, branched or unbranched, or becomes swollen. The germinating zoospore, in the course of six to twenty-four hours, commonly bears a sporangium at the end of its germ-tube (Fig. 1). This mode of germination has been observed by Raciborski[3] in *Phytophthora Colocasiæ* and by me in *Ph. parasitica* on castor. The sporangia borne on germ-tubes are pear-shaped and measure 13—20 × 10—15μ. They germinate either by discharging their zoospores, which are from 2 to 6 in number and as big as those produced by sporangia from nutrient media, or by giving rise to germ-tubes or to secondary sporangia.

Resting conidia resemble those of *Ph. parasitica* in all respects, except in size ; the former are smaller, measuring 10—42μ in diameter.

According to Klebs,[4] *Oedogonium* can be readily induced to form antheridia by regulating light, amount of water and the nutrient quality of the medium used. It has not been found possible to induce the development of sexual organs of this fungus by any systematic method, for it is exceedingly difficult to determine the predisposing factors. Oospores were found first in one of the six French bean juice agar tubes and a fortnight later in another, all of which had been inoculated the same day with mycelium from a culture growing on Quaker-Oat agar. These six French bean juice agar tubes belonged

[1] Butler, E. J. and Kulkarni, G. S. Studies in *Peronosporaceæ*. *Mem. Dept. Agric. India, Bot. Ser.* V, No. 5, 1913.

[2] Wilcox, E. M., Link, G. K. and Pool, V. W. A dry rot of the Irish Potato tuber. *Nebraska Agri. Expt. Sta. Res. Bull.* No. 1, 1913.

[3] Raciborski, M. *Parasitische Algen und Pilze Java's* I, 1900.

[4] Klebs, G. *Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen*, 1896.

to the same batch. Evidently therefore there can hardly be any difference in the composition of their contents. Before they were inoculated they had

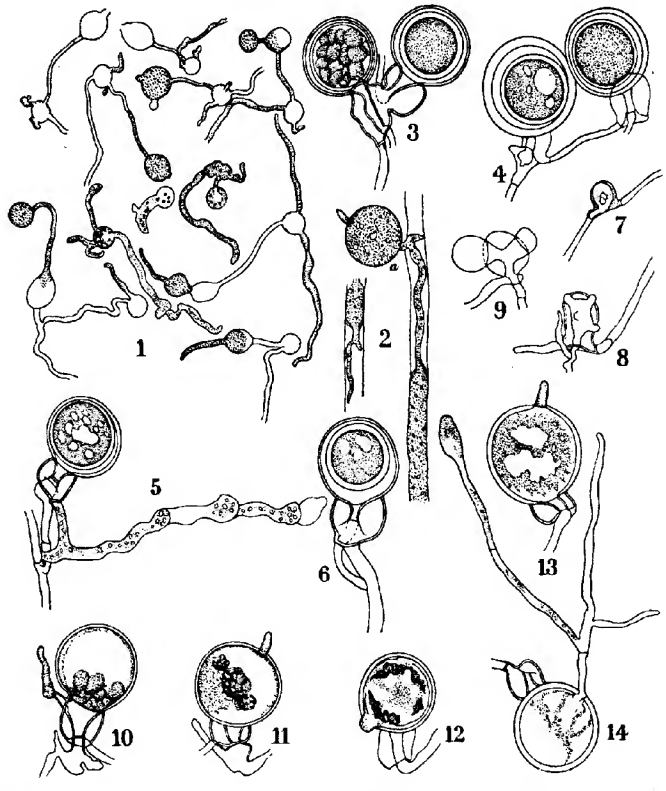

Fig. 1. Zoospores germinating and producing sporangia and secondary sporangia × 305.

Fig. 2. Branches arising through septa.
(*a*) shows the formation of a sporangium on such a branch × 305.

Fig. 3. Double antheridium × 557.

Fig. 4. Branched antheridium × 557.

Fig. 5. An oospore, the oogonium and antheridium of which arise from the same parent hypha.
The oogonium and antheridium are lateral in origin × 557.

Fig. 6. An oospore, the oogonial stalk arises laterally from the antheridial stalk × 557.

Fig. 7. An oogonium originating from within an antheridium × 557.

Fig. 8. An oogonium piercing an antheridium on its way out at several points × 557.

Fig. 9. An oogonium bifurcating within an antheridium × 557.

Fig. 10. An oogonium germinating vegetatively × 557.

Figs. 11—14. Germinating oospores × 557.
In Figs. 11, 13 and 14 the oospore wall has completely dissolved.
In Fig. 12 the oospore wall is partly dissolved and what is left of it is wavy in outline.

been placed in the same basket and after inoculation they were all in the same jar exposed to light. It is therefore difficult to understand why only two of these cultures formed oospores while the remaining four developed only sporangia and resting conidia. Subcultures were made on French bean juice agar, Oat juice agar, Quaker-Oat agar and Wheat juice agar from these cultures bearing oospores. Of these subcultures only one on Oat juice agar developed oospores and although over a couple of dozen cultures were made on Quaker-Oat agar from different sources still only one tube of this medium gave oospores. Many cultures on Oat juice agar produced oospores but their development had no relation to the parent cultures. Tubes inoculated with cultures producing oospores did not necessarily bear the sexual organs while transfers made from cultures on the same medium and from French bean juice agar cultures not bearing oospores occasionally produced them. Thus it is not easy to say what factor or factors stimulate the development of oospores. This discontinuous production of oospores differs from the result got by Pethybridge and Murphy[1] with *Ph. infestans* (Mont.) de Bary, where a culture once having commenced to form oospores, continues to do so without break in the subsequent transfers.

The development of oospores follows the method found almost simultaneously in five species of this genus, *viz.*, *Ph. erythroseptica* Pethyb., *Ph. infestans* (Mont.) de Bary, *Ph. Phaseoli* Thaxt., *Ph. parasitica* Dast. and *Ph. Colocasiæ* Rac. Antheridia and oogonia are always produced embedded in the nutrient medium, and are as a rule borne on separate hyphæ, but in a few cases they have been found to be borne on a common stalk (Fig. 6).

The antheridium of the *Phytophthora* upon *Vinca* is identical with that of the castor parasite; in certain cases branched and double antheridia (Figs. 4 and 3) were observed in the fungus upon *Vinca*.

Whenever it has been possible to trace clearly the oogonial stalk for some distance it has been found that, like the antheridial stalk, it has a lateral origin (Fig. 5). In a few cases the oogonium has been observed to be intercalar, so also the antheridium. The oogonium has also been found to arise from within the antheridium as in *Ph. parasitica* (Fig. 7). The tentative oogonial incept is at first thin-walled and is distinguishable from the vegetative branches of the hyphæ by its having dense and coarsely granular protoplasm; when it comes in contact with an antheridium its apex as a rule swells before piercing the antheridial wall. It generally enters the antheridium somewhere at or near the base, as in the other species belonging to the "*infestans*-group."

[1] Pethybridge, G. H., and Murphy, P. A. *loc. cit.*

The swollen or knob-like portion of the oogonial incept that is outside the antheridial incept may give out a sterile projection like the antheridium. In a few cases two oogonial incepts have been found to penetrate the same antheridium at two different points, but these oogonial incepts have never been found to mature ; they were never observed to break through the antheridium. It is quite possible for more than one oogonium to be attracted towards a single antheridium and to make their way within it ; but it is a question whether the antheridium would be able to fertilize more than one oogonium. The oogonial incept within the antheridium is club-shaped, grows within it and eventually bursts through it at some point. In an exceptional case the oogonial incept within the antheridium had made attempts to break through at four places by means of four projections three of which had succeeded in boring their way out (Fig. 8). In another case the oogonial incept within the antheridium bifurcated before leaving it and two branches made their way out by piercing the antheridial wall at two different points (Fig. 9). It seems improbable that these oogonia would have matured and produced oospores. After leaving the antheridium, the oogonial incept swells out into a globose body, the oogonium proper. The course of development of the oospore could not be observed. For the asexually formed sporangia to revert to the vegetative condition is common and cases have also been known in some species of *Pythium*, where the oogonium had also reverted to the vegetative condition. Thus Wahrlich[1] has observed oogonia, which, on being not fertilized, continue their growth vegetatively. Ward[2] has also made like observations. A similar case has been found in the fungus under study (Fig. 10). An oogonium in Oat juice agar, after making its way out from within the antheridium, had grown to its full size but the wall was still unthickened and uncoloured ; it was almost empty of its protoplasmic contents, except the thin layer that lined the inside of the oogonial wall and the little mass that was at the base of the oogonium. Within the antheridium a lateral branch had grown out piercing the antheridial wall. Outside the antheridium it became septate and the portion beyond the septum had protoplasmic contents finely granulated and was thin-walled, like an ordinary hypha, while that below the septum was empty and slightly thicker, as thick as the oogonium from which it had arisen ; it also contained a small cellulose ingrowth. The oogonium on failing to form an oospore had thus germinated vegetatively. The colour, thickness, and size of the oogonial wall is influenced by the medium

1 Wahrlich, W. *Pythium* n. sp. *Ber. Deutsch. Bot. Ges.*, V, 1887.

2 Ward, H. M. Observations on the genus *Pythium*. *Quart. Journ. Micros. Science*, XXIII (N. S.), 1883.

in which it is growing, as in *Phytophthora* on castor ; but very often on the same medium smooth and rough-walled oospores have been produced, the development of secondary thickening depending upon local conditions. In French bean juice agar the oogonium is very slightly yellow tinted, almost hyaline ; in Oat juice agar and Quaker-Oat agar it is honey-coloured ; in the latter medium of a lighter colour than in the former.

The size of the oospore, unlike that of the *Phytophthora* on castor, is also influenced by the medium in which it is grown. In French bean juice agar it varies from 14—18μ in diameter, extreme measurements being 11μ and 20μ and the average of 97 measurements being 15·8μ ; in Oat juice agar and in Quaker-Oat agar from 16—21μ in diameter, extreme measurements being 15μ and 23μ and the average of 57 measurements being 18·4μ ; the size of the oospores on these last two media closely agrees with that of oospores of the castor fungus. Generally the wall of the oospore is not very thick, but it seems that it is inversely proportional to the thickness of the surrounding oogonial wall. In French bean juice agar, it is slightly thicker than in Oat juice agar or Quaker-Oat agar. The oospore is hyaline in colour.

Attempts were made to germinate oospores in tap water and sterilized water taken from wayside pools but they failed, while a few of the many oospores from Oat juice agar sown in wet earth showed attempts at germination. It appears that the germination is preceded by the disappearance of the oil globule or globules and by the fine protoplasmic contents of the oospores turning coarser. The germination is accompanied by the complete or partial dissolution of the oospore wall (Figs. 11 to 14). When the oospore is just beginning to germinate its wall shows at times a zig-zag outline due to its unequal dissolution (Fig. 12), as observed by Pethybridge[1] in the germinating oospores of *Ph. erythroseptica* Pethyb. In one case the germ-tube had grown into a long branched hypha, the end of one of the branches being swollen as if it was going to form a sporangium (Fig. 14) ; but further growth did not take place as the hypha became empty of its contents. From the few cases observed it seems that the colour of the oogonium of the germinating oospore growing on Oat juice agar becomes lighter. Two oospores in an Oat juice agar tube were found to have produced short germ-tubes, one each. In both these cases the oospore wall had completely dissolved, and the oogonium was partly empty. In one a thin layer of protoplasm was lining the inside of the oogonial wall. No oil globules were found within the two germinating oospores and the contents were coarsely granular. As far as could be judged

1 Pethybridge, G. H. Further observations on *Ph. erythroseptica* Pethyb. and on the disease produced by it in the potato plant. *Sc. Proc. Roy. Dub. Soc.*, XIV (N.S.), No. 10, 1914.

from the few oospores of the *Phytophthora* on *Vinca rosea*, that have been observed germinating, it appears that the mode of germination agrees, at least in essential points, with that of *Ph. erythroseptica* described minutely by Pethybridge.

On account of the peculiar mode of development of oospores, this fungus belongs to what Pethybridge[1] calls the "*infestans*-group." Of all the species of this group, the *Phytophthora* on *Vinca rosea* is most closely allied to *Ph. parasitica* Dast. on castor. Inoculation experiments were carried out to trace further the affinity between these two. From the very commencement of the study of this parasite it was found that, in order to get successful results, the inoculated plant, even if it be the original host, must be kept in an atmosphere saturated with moisture. Inoculated plants were, therefore, always kept in such an atmosphere as described on page 233. It may be noted that no such precautions were required with plants successfully inoculated by *Ph. parasitica*, and that the negative results of the inoculation of certain plants detailed below with the *Phytophthora* on castor have been obtained even when they had been tried under very moist conditions. Inoculations on living plants were in all cases made by means of zoospores from pure cultures.

The following table shows the results of various inoculation experiments :—

Name of host	Results of inoculation with *Phytophthora* on *Vinca*	Results of inoculation with *Phytophthora* on castor
Clarkia elegans. Seedlings in a pot ..		
Gilia nivalis and mixed species. Seedlings in a pot.		
Salpiglosus variabilis and mixed species. Seedlings in a pot.		
Schizanthus retusa and mixed species. Seedlings in a pot.	Positive	Positive
Ricinus communis. Seedlings in a pot ..		
Syringa vulgaris. Small plants.		
Vinca rosea. Mature plants		
Fruits of *Vinca rosea*	Positive	Negative

1 Pethybridge, G. H. On the Rotting of Potato Tubers by a New Species of *Phytophthora* having a Method of Sexual Reproduction hitherto undescribed. *Sc. Proc. Roy. Dub. Soc.*, XIII (N. S.), No. 35, 1913.

Name of host	Results of inoculation with *Phytophthora* on *Vinca*	Results of inoculation with *Phytophthora* on castor
Fruits of *Ricinus communis*	Positive	Positive
Petunia sp. Seedlings in a pot	Positive	Negative
Martynia diandra. Mature plants		
These two hosts have been found in nature to be attacked by a *Phytophthora*.		
Solanum melongena. Young plants ..	Negative	Positive.
Solanum tuberosum. Young plants ..		
Lycopersicum esculentum. Young plants ..	Positive	Positive.
Living potato tubers (inoculated through wounds).	Positive (poor growth)	Positive (copious growth)
Sterilized corms of *Colocasia antiquorum* ..	Positive	Negative.
Sterilized potatoes	Negative	Positive.
Sterilized ants		

In the author's opinion, these differences and those found from the study of pure cultures are not enough to justify the making of a new species, of the *Phytophthora* on *Vinca rosea*. Cumulative evidence shows that it is only a biologic variety of *Phytophthora parasitica* Dast. on castor.

SUMMARY.

1. A weak parasite belonging to the genus *Phytophthora* was found attacking *Vinca rosea* in May, 1913, under very wet climatic conditions.

2. Microscopic characters of this disease in *Vinca* are similar to those of *Ph. parasitica* Dast. on castor except that sporangia are borne on both the surfaces and that sporangiophores are smaller.

3. Asexual spores agree with those of *Ph. parasitica;* the chief differences are that resting conidia are smaller and zoospores produce as many as four germ-tubes while those of *Ph. parasitica* have not been observed to produce more than two.

4. The formation of oospores is identical with that of *Ph. parasitica.* The size of the oospore like that of the oogonium is influenced by the medium in which it grows. On certain media (Oat juice agar and Quaker-Oat agar) the measurements agree closely with those of *Ph. parasitica* from castor. From the few cases of the germination that have been observed it appears that the mode of germination is the same as that observed by Pethybridge in *Ph. erythroseptica* Pethyb.

5. Inoculation experiments show that there are some points of difference between the *Phytophthora* on castor and that on *Vinca.* The latter is distinctly weaker in parasitism than the former. Some of the hosts are common to both, while some which were attacked by the *Vinca* parasite resisted infection by that from castor.

6. It is concluded that the fungus is not a distinct species but only a biologic strain of *Ph. parasitica* Dast.

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